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Research Note

Development of Challenge Models To Evaluate the Efficacy of a Vaccine To Reduce Carriage of *Salmonella* in Peripheral Lymph Nodes of Cattle†

T. S. EDRINGTON,[1]* G. H. LONERAGAN,[2] J. HILL,[1] K. J. GENOVESE,[1] D. M. BRICHTA-HARHAY,[3] R. L. FARROW,[1] N. A. KRUEGER,[1] T. R. CALLAWAY,[1] R. C. ANDERSON,[1] AND D. J. NISBET[1]

[1]*U.S. Department of Agriculture, Agriculture Research Service, Southern Plains Agriculture Research Center, Food and Feed Safety Research Unit, 2881 F&B Road, College Station, Texas 77845;* [2]*Department of Animal and Food Sciences, P.O. Box 42141, Texas Tech University, Lubbock, Texas 79409; and* [3]*U.S. Department of Agriculture, Agriculture Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, Nebraska 68933, USA*

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ABSTRACT

Because challenge models to infect peripheral lymph nodes (PLNs) with *Salmonella* have not been reported, we performed a series of experiments to develop and refine challenge models to evaluate an intervention applied at the animal level and to provide initial estimates of efficacy of an intervention (i.e., a vaccine) to aid in the design of future studies. In each of four experiments, steers (control or vaccinated) were inoculated with *Salmonella* strains Montevideo or Newport, and in experiment IV, *Salmonella* Senftenberg was also used. Calves were euthanized 14 to 42 days postinoculation, and PLNs were collected. In the first experiment, calves were challenged with $\sim 10^{10}$ *Salmonella* cells, and few treatment differences were observed 14 days postchallenge. However, by day 21, *Salmonella* Newport was recovered from fewer vaccinated calves than control calves ($P < 0.05$). In experiment II, calves were challenged with $\sim 10^{7}$ *Salmonella* cells and, after two necropsies (14 and 28 days postchallenge), only one lymph node was *Salmonella* positive; therefore, the study was terminated. In experiment III, calves were again challenged with $\sim 10^{10}$ *Salmonella* cells, and no significant effect of vaccine was observed in calves challenged with Montevideo or Newport strains. A transdermal route of challenge was explored in experiment IV, using a 10-lancet, allergy testing instrument. Sixteen steers were challenged with either *Salmonella* Newport or *Salmonella* Montevideo (*Salmonella* Newport right legs; *Salmonella* Montevideo left legs), and all steers were challenged on the lower abdomen with *Salmonella* Senftenberg. Transdermal inoculation resulted in predictably *Salmonella*-positive PLNs, and a modest vaccine effect was detected. Because it is well tolerated by the calves and results in predictable and regionally specific *Salmonella* recovery from PLNs, the transdermal route of challenge may be preferred by researchers wishing to evaluate the impact of interventions designed to reduce the carriage of *Salmonella* in PLNs.

Recent research suggests that *Salmonella* may be commonly harbored in peripheral lymph nodes (PLNs) of cattle presented for harvest *(1, 7, 10)*. Because PLNs are frequently included in ground beef, *Salmonella* carriage in PLNs likely results in some degree of *Salmonella* contamination of ground beef. It may be practical to remove large, easily accessible PLNs during harvest; however, it is impractical to remove all PLNs, as cattle have many small PLNs throughout their carcasses. It is possible that preharvest control of *Salmonella* may complement within-plant control efforts and reduce the likelihood of ground beef contamination.

A vaccine containing siderophore receptors and porin proteins from *Salmonella* Newport was associated with reduced shedding of *Salmonella* in the feces of dairy cattle *(6, 11)*. In another study *(9)* of this vaccine, no difference in fecal *Salmonella* prevalence was observed, although the *Salmonella* prevalence and study design did not lend itself to such a comparison. No differences were observed in fecal shedding of *Salmonella* in studies of feedlot cattle *(2)* or dairy cows *(8)*. It is possible, however, that immunity against *Salmonella* may reduce the duration of infection within lymph nodes regardless of an effect, or lack thereof, within the lumen of the intestine. If so, this vaccine may reduce the prevalence of *Salmonella* within the PLNs of cattle presented for harvest.

Because challenge models to infect PLNs with *Salmonella* have not been reported, we performed a series of four experiments to develop and refine challenge models that can be used to evaluate an intervention applied at the animal level and to provide initial estimates of efficacy of an intervention (i.e., a vaccine) that can be used by researchers to aid the design of future studies.

MATERIALS AND METHODS

Care, use, and handling of experimental animals were preapproved by the Animal Care and Use Committee of the Food and Feed Safety Research Laboratory, U.S. Department of

* Author for correspondence. Tel: +1 979-260-3757; Fax: +1 979-260-9332; E-mail: tom.edrington@ars.usda.gov.

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Agriculture. Recently weaned Holstein and Holstein-cross steers were purchased from a single supplier and transported to our laboratory in College Station, TX. Upon arrival, steers were weighed, identified with an ear tag, and maintained in a large outside lot and fed a commercial nonmedicated calf starter and grass hay. In experiment I, symptoms of bovine respiratory disease were observed in most steers, and all were administered enrofloxacin (Baytril 100, Bayer Animal Health LLC, Shawnee Mission, KS). In subsequent experiments, steers were metaphylactically administered tulathromycin (Draxxin, Pfizer Animal Health, New York, NY) per manufacturer's recommendations upon arrival. Rectal swabs were collected weekly prechallenge and were cultured for *Salmonella*.

Following acclimation (3 to 5 weeks), steers were randomly assigned to treatment (control or vaccine). Vaccinated steers were administered a commercially available *Salmonella* vaccine on days 0 and 21 per label directions (2 ml subcutaneous; *Salmonella* Newport Bacterial Extract vaccine with SRP Technology, Pfizer Animal Health, Madison, NJ), whereas control animals received a sham injection of corn oil (2 ml subcutaneous). Steers were housed outdoors in covered, concrete floored pens, either two or four steers per pen, and were fed a diet to meet or exceed their nutritional requirements. Pens were washed daily. Steers were euthanized (Euthasol, Delmarva Laboratories, Midlothian, VA), and the right and left subiliacs, popliteals, and superficial cervical nodes were collected, weighed, and cultured for the challenge strains of *Salmonella* as described below.

Experiment I. Thirty-two steers (average body weight 81 kg) were inoculated with either *Salmonella* Montevideo or *Salmonella* Newport in a 2 × 2 factorial design such that there were eight calves per treatment. Calves were challenged orally with 20 ml of tryptic soy broth (TSB) containing 1.0 and 1.2 × 10^{10} CFU of *Salmonella* Montevideo or *Salmonella* Newport, respectively. Body weights were collected weekly throughout the experimental period. Fourteen days postchallenge, one-half of the calves in each pen (and treatment) were euthanized and PLNs were collected. At 21 days postchallenge, all remaining calves were euthanized and PLNs were collected.

Experiment II. The design was similar to that of experiment I, except that the oral challenge included 4.2 and 6.0 × 10^{7} CFU of *Salmonella* Montevideo and *Salmonella* Newport, respectively. Two calves per treatment were necropsied 14 and 28 days postchallenge. Due to the poor recovery of *Salmonella* from the PLNs, the study was terminated.

Experiment III. Using a design similar to that of experiments I and II, calves were challenged with 1.5 and 1.3 × 10^{10} CFU of *Salmonella* Montevideo and *Salmonella* Newport, respectively, in 20 ml of TSB, and two calves per treatment were necropsied on days 14, 28, 35, and 42 postchallenge. In addition to the nodes described above, axillary lymph nodes (right and left) were collected.

Experiment IV. Sixteen steers (average body weight 193 kg; two per pen by treatment) were randomly allocated to vaccine or control treatment. Calves were challenged with either *Salmonella* Newport (7.9 × 10^{8}/ml; eight steers) or *Salmonella* Montevideo (1.2 × 10^{9}/ml; eight steers) using a 10-lancet allergy testing instrument (ComforTen Multiple Skin Test Device, Hollister-Stier Allergy, Spokane, WA) as described elsewhere *(5)*. Four applications of this 10-lancet instrument were made to each leg; two applications were medial and two were lateral to the metacarpus-metatarsus, such that *Salmonella* Newport was challenged in the right legs and *Salmonella* Montevideo in the left legs. Additionally, all calves were challenged on the lower abdomen with *Salmonella* Senftenberg (4.3 × 10^{8}/ml) via two applications each on the right and left sides. A new instrument was used for the different serovars and for each calf. Three and 6 days following *Salmonella* challenge, one-half of the calves in each treatment were euthanized and PLNs were collected.

Lymph node processing. Within 15 min of collection, lymph nodes were transferred to the laboratory and each node was trimmed of excess fat and fascia. Trimmed lymph nodes were weighed and then surface sterilized by immersion in boiling water for 3 s. The sterilized lymph node was placed into a filtered stomacher bag, and the tissue was pulverized using a rubber mallet. Tetrathionate broth (20 ml) was added to each sample bag, followed by mixing for 60 s with a laboratory blender. For quantitative culture, 1 ml of the pulverized lymph node–tetrathionate broth mixture was removed and 50 μl was direct plated on xylose lysine deoxycholate agar using a commercially available spiral plater (Spiral Biotech Autoplate 4000, Advanced Instruments, Inc., Norwood, MA). Plates were incubated (37°C, 24 h) followed by an additional 24 h at room temperature. Black colonies were counted and converted to log CFU per gram PLN. Following spiral plating, an additional 80 ml of tetrathionate broth was added, and the lymph node–tetrathionate mixture was incubated overnight (37°C). A sample (100 μl) of this enrichment was transferred to 5 ml of Rappaport-Vassiliadis broth and incubated at 42°C for 24 h, and then it was plated for isolation on brilliant green agar supplemented with novobiocin (25 μg/ml). Plates were incubated at 37°C overnight, and *Salmonella* isolates were serogrouped (three isolates per PLN). Serogrouping was conducted using slide agglutination with *Salmonella* antiserum (Difco, BD, Detroit, MI). Rectal swabs were enriched in 20 ml of tetrathionate broth and were incubated at 37°C overnight; next, 100 μl was inoculated into 5 ml of Rappaport-Vassiliadis broth, incubated as above, and then plated for isolation on brilliant green agar supplemented with novobiocin and incubated as described.

Statistical analysis. Data were analyzed using SAS software (version 9.3, SAS Institute Inc., Cary, NC). Contingency tables were developed and within-table dependency was evaluated using either a chi-square statistic or a Fisher's exact test. Logistic regression models were constructed to compare treatment effects.

RESULTS

Rectal swabs collected prechallenge were all *Salmonella* negative except for experiment III, in which a few swabs were positive and all of the isolates belonged to serogroups different from the challenge strains. In experiment I, *Salmonella* was recovered from 58.3 and 87.5% of PLNs and calves, respectively. No significant differences were observed in the percentage of PLNs positive for *Salmonella* Montevideo or *Salmonella* Newport on day 14 (Table 1). At 21 days postinoculation, *Salmonella* Newport was recovered from fewer ($P < 0.05$) PLNs among the vaccinated calves (4%) compared with the control calves (54%). With two exceptions, all recovered isolate serogroups matched the respective challenge strains. Two steers in the Montevideo (serogroup C_1) treatment (one each control and vaccine) also had serogroup C_2 isolates cultured from their lymph nodes.

TABLE 1. *Prevalence of* Salmonella *serovars (Montevideo and Newport) in the peripheral lymph nodes of vaccinated or control calves necropsied 14 or 21 days postchallenge (experiment I)*[a]

	14 days postchallenge ($n = 16$)				21 days postchallenge ($n = 16$)			
	Montevideo		Newport		Montevideo		Newport	
Lymph node	Control	Vaccine	Control	Vaccine	Control	Vaccine	Control	Vaccine
Subiliac								
Right	50	75	50 A	100 B	100	75	25	0
Left	50	75	75	100	100	75	50	25
Popliteal								
Right	50	75	50	50	25	75	75 C	0 D
Left	50	50	50 A	100 B	100	75	50 A	0 B
Superficial cervical								
Right	50	75	50 A	100 B	75	75	75 C	0 D
Left	50	50	50	50	50 C	100 D	50 A	0 B
All nodes	50	67	54 A	83 B	75	79	54 C	4 D

[a] Vaccine, administered a commercially available *Salmonella* vaccine; Control, administered a sham injection. Values followed by letters A and B indicate that row percentages within necropsy and *Salmonella* strain tend to differ ($P < 0.10$); values followed by letters C and D indicate that row percentages within necropsy and *Salmonella* strain are different ($P < 0.05$).

In experiment II, *Salmonella* was only recovered from two PLNs harvested during the first two necropsies (14 and 28 days postinoculation); therefore, the study was terminated. The higher challenge dose (i.e., $\sim 10^{10}$) in experiment III resulted in the recovery of *Salmonella* from PLNs. *Salmonella* was recovered from 35.2 and 62.5% of PLNs and calves, respectively. No significant treatment differences were observed, with one exception: the vaccine treatment decreased ($P < 0.05$) the percentage of *Salmonella*-positive left axillary nodes compared with controls across serotypes. *Salmonella* was recovered from fewer PLNs of calves challenged with *Salmonella* Newport than from those challenged with *Salmonella* Montevideo (Table 2). The majority of isolates (98%) matched the serogroup of the challenge strain. The only exceptions were that *Salmonella* Montevideo was cultured from the popliteal and subiliac in one calf on day 35 and from the subiliac in another calf on day 42; both of these calves were inoculated with *Salmonella* Newport.

TABLE 2. *Prevalence of* Salmonella *(Montevideo and Newport) in the peripheral lymph nodes of vaccinated or control calves (experiment III)*[a]

	Montevideo		Newport	
Lymph node	Control	Vaccine	Control	Vaccine
Subiliac				
Right	75	87.5	25	0
Left	75	62.5	25	12.5
Popliteal				
Right	50	62.5	12.5	12.5
Left	37.5	62.5	0	12.5
Superficial cervical				
Right	50	75	0	0
Left	50	75	0	0
Axillary				
Right	50	62.5	0	0
Left	87.5	50	12.5	0
All nodes	56.3	70.8	10.4	6.3

[a] Vaccine, administered a commercially available *Salmonella* vaccine; Control, administered a sham injection.

In the transdermal challenge model (experiment IV), *Salmonella* was recovered from 58.3 and 93.8% of PLNs and calves, respectively. No treatment differences were observed among calves inoculated with *Salmonella* Montevideo, except that there was reduced ($P < 0.05$) likelihood of recovery from the right subiliac lymph nodes among vaccinates compared with controls (Table 3). Across all nodes, the likelihood of recovery of *Salmonella* Newport from PLNs was lower ($P = 0.03$) among vaccinated calves (33.3%) compared with controls (66.7%). All but one isolate matched the serogroup of regional challenge. The only exception was that one isolate from a subiliac lymph node was serogroup C_2 (presumably Newport) instead of E_4 (i.e., Senftenberg).

DISCUSSION

In the work described herein, we developed two distinct routes of *Salmonella* challenge that resulted in *Salmonella* recovery from PLNs. Because prevalence of *Salmonella* in PLNs is a function of incidence (i.e., rate of new PLN infections) and duration of infection, we included various windows of harvest to capture a change in the duration of infection, given that we attempted to control the incidence (i.e., by providing the challenge at one time point). In experiment I, the oral challenge, no evidence of a reduction in prevalence was observed 14 days after challenge. After 21 days, a decrease was observed in calves challenged with *Salmonella* Newport, which likely indicated an increased rate of clearance (or reduced duration of infection). Also, a treatment effect was observed in experiment IV (transdermal),

TABLE 3. *Prevalence of* Salmonella*-positive lymph nodes in vaccinated or control calves following transdermal challenge of* Salmonella *to the lower legs and ventral abdomen (experiment IV)*[a]

Node	Montevideo/Senftenberg		Newport/Senftenberg		Combined strains	
	Control	Vaccine	Control	Vaccine	Control	Vaccine
Subiliac						
Right	75 A	0 B	25	25	50	12.5
Left	0	25	75	25	38	25
Popliteal						
Right	75	100	75	50	75	75
Left	75	75	50	25	63	50
Superficial cervical						
Right	75	100	75	75	75	88
Left	100	100	100 A	0 B	100 A	50 B
All nodes	67	67	67 C	33 D	67	50

[a] *Salmonella* strains Montevideo and Newport ($n = 16$ calves each) were administered to the lower legs; *Salmonella* Senftenberg (all calves) was administered to the ventral abdomen. Vaccine, administered a commercially available *Salmonella* vaccine; Control, administered a sham injection. Values followed by letters A and B indicate that row percentages within *Salmonella* strain are different ($P < 0.05$); values followed by letters C and D indicate that row percentages within *Salmonella* strain tend to differ ($P \leq 0.10$).

and a numerical reduction was observed in experiment III. Despite this evidence supporting its efficacy against *Salmonella* Newport, no association (even with a liberal interpretation of *P* values) was observed for *Salmonella* Montevideo. This may be because there is a lack of antigenic homology between the challenge serotypes or because Montevideo has additional mechanisms for iron acquisition, or it may be due to other variations among host-bacteria interactions.

It is clear from the work described herein that a substantial oral dose (i.e., $\sim 10^{10}$) of *Salmonella* is required to result in recovery of *Salmonella* from PLNs. In experiment II, the lower dose failed to produce *Salmonella* in PLNs at detectable concentrations. On occasion, we did recover serogroups other than the challenge serogroup. It may be that repeated lower doses would have been equally effective as (or even more effective than) a single large challenge. Whereas repeated exposures may better mimic real-world events, we attempted to control incidence to the extent possible so that observed differences in vaccine status (or in serotype status) were primarily a reflection of changes in duration of infection. Once duration of infection is known for specific serotypes, variation in challenge regimens might be explored.

The recovery of serogroup C_2 in calves challenged with *Salmonella* Montevideo (i.e., C_1) (experiment I) and of C_1 in calves challenged with *Salmonella* Newport (experiment III) may have resulted from cross-contamination via workers, flies, birds, air movement, or the environment. Alternatively, we cannot rule out prior exposure, as these serotypes are frequently isolated from dairy cattle *(3, 4, 12)*. As described elsewhere *(5),* we hypothesize that a transdermal route of infection accounts for some *Salmonella* recovered from the PLNs of cattle presented for harvest. Consequently, herein we report an initial extension of a transdermal route of infection *(5)* as the experiment IV challenge study. Multiple serotypes were used within the sample animal (i.e., Senftenberg and Montevideo or Senftenberg and Newport), and this route of challenge predictably resulted in positive PLNs. Moreover, the serogroups recovered from the PLNs that drain the challenge region (e.g., right foreleg to prescapular lymph node versus ventral abdomen to subiliac lymph node) matched in all but one instance. Similar to experiment I, a vaccine effect was observed for *Salmonella* Newport but not for *Salmonella* Montevideo.

Across all necropsy days, the relative magnitude of association between vaccine status and *Salmonella* Newport prevalence for experiments I, III, and IV was 20.3, 39.4, and 50%, respectively. These data, in conjunction with the control prevalence, should inform the design and sample size calculations of future studies. While the ideal window in which to sample PLNs subsequent to challenge is not completely certain, the time periods described herein provide a reasonable estimate.

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REFERENCES

1. Arthur, T. M., D. M. Brichta-Harhay, J. M. Bosilevac, M. N. Guerini, N. Kalchayanand, J. E. Wells, S. D. Shackelford, T. L. Wheeler, and M. Koohmaraie. 2008. Prevalence and characterization of *Salmonella* in bovine lymph nodes potentially destined for use in ground beef. *J. Food Prot.* 71:1685–1688.
2. Dodd, C. C., D. G. Renter, D. U. Thomson, and T. G. Nagaraja. 2011. Evaluation of the effects of a commercially available *Salmonella* Newport siderophore receptor and porin protein vaccine on fecal shedding of *Salmonella* bacteria and health and performance of feedlot cattle. *Am. J. Vet. Res.* 2:239–247.
3. Edrington, T. S., T. R. Callaway, R. C. Anderson, and D. J. Nisbet. 2008. Prevalence of multidrug-resistant *Salmonella* on commercial

dairies utilizing a single heifer raising facility. *J. Food Prot.* 71:27–34.

4. Edrington, T. S., B. H. Carter, T. H. Friend, G. R. Hagevoort, T. L. Poole, T. R. Callaway, R. C. Anderson, and D. J. Nisbet. 2009. Influence of sprinklers, used to alleviate heat stress, on faecal shedding of *E. coli* O157:H7 and *Salmonella* and antimicrobial susceptibility of *Salmonella* and *Enterococcus* in lactating dairy cattle. *Lett. Appl. Microbiol.* 48:738–743.
5. Edrington, T. S., G. H. Loneragan, J. Hill, K. J. Genovese, H. He, T. R. Callaway, R. C. Anderson, D. M. Brichta-Harhay, and D. J. Nisbet. 2013. Development of a transdermal *Salmonella* challenge model in calves. *J. Food Prot.* 76:1255–1258.
6. Farrow, R. L. 2012. Quantitative herd-level evaluation of a commercially available vaccine for control of *Salmonella* in dairy cattle. Ph.D. dissertation. Texas A&M University, College Station.
7. Haneklaus, A. N., K. B. Harris, D. B. Griffin, T. S. Edrington, L. M. Lucia, and J. W. Savell. 2012. *Salmonella* prevalence in bovine lymph nodes differs among feedyards. *J. Food Prot.* 75:1131–1133.
8. Heider, L. C., R. W. Meiring, A. E. Hoet, W. A. Gebreyes, J. A. Funk, and T. E. Wittum. 2008. Evaluation of vaccination with a commercial subunit vaccine on shedding of *Salmonella enterica* in subclinically infected dairy cows. *J. Am. Vet. Med. Assoc.* 233:466–469.
9. Hermesch, D. R., D. U. Thomson, G. H. Loneragan, D. R. Renter, and B. J. White. 2008. Effects of a commercially available vaccine against *Salmonella enterica* serotype Newport on milk production, somatic cell count, and shedding of *Salmonella* organisms in female dairy cattle with no clinical signs of salmonellosis. *Am. J. Vet. Res.* 9: 1229–1234.
10. Koohmaraie, M., J. A. Scanga, M. J. De La Zerda, B. Koohmaraie, L. Tapay, V. Beskhlebnaya, T. Mai, K. Greeson, and M. Samadpour. 2012. Tracking the sources of *Salmonella* in ground beef produced from nonfed cattle. *J. Food Prot.* 75:1464–1468.
11. Loneragan, G. H., D. U. Thomson, R. M. McCarthy, H. E. Webb, A. E. Daniels, T. S. Edrington, D. J. Nisbet, S. J. Trojan, S. C. Rankin, and M. M. Brashears. 2012. *Salmonella* diversity and burden in cows on and culled from dairy farms in the Texas high plains. *Foodborne Pathog. Dis.* 9:549–555.
12. U.S. Department of Agriculture, Food Safety and Inspection Service. 2010. Progress report on *Salmonella* testing of raw meat and poultry products, 1998–2010. Available at: www.fsis.usda.gov/PDF/Salmonella_Progress_Report_1998-2003.pdf. Accessed 10 April 2013.